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Factorial Trial of Three Interventions to Reduce the Progression of Precancerous Gastric Lesions in Shandong, China: Design Issues and Initial Data

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ABSTRACT: In the fall of 1995, 3411 subjects in 13 rural villages in Linqu County, Shandong Province, China, began participating in a blinded, randomized 2^3 factorial trial to determine whether interventions can reduce the prevalence of dysplasia and other precancerous gastric lesions. One intervention is treatment for infection by *Helicobacter pylori* with amoxicillin and omeprazole. A second is dietary supplementation with capsules containing vitamin C, vitamin E, and selenium. A third is dietary supplementation with capsules containing steam-distilled garlic oil and Kyolic aged garlic extract. Investigators will evaluate histopathologic endpoints after gastroscopies with biopsies from seven standard sites in 1999. Initial data from pill counts and sampled blood levels of vitamin E, vitamin C, and S-allylcysteine indicate excellent compliance. Subjects have tolerated all interventions well, although 3.1% of those assigned to amoxicillin and omeprazole developed rashes, compared to 0.3% to those in the control group. Preliminary breath

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tests demonstrate substantial reductions in gastric urease activity, an indication of infection by *Helicobacter pylori*, among those assigned to amoxicillin and omeprazole. *Controlled Clin Trials* 1998;19:352–369 © Elsevier Science Inc. 1998

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INTRODUCTION

Gastric cancer (GC) is the second most common cancer worldwide [1] and the most common cancer in China [2]. Since 1983, the National Cancer Institute (NCI) has collaborated with the Beijing Institute for Cancer Research (BICR) on studies to define the epidemiology and etiology of GC in Linqu County, Shandong Province, China, where GC accounts for 42% of deaths from cancer [2].

Studies in Linqu have demonstrated the ubiquity of precursor lesions to gastric cancer. A gastroscopic survey with biopsies in 1989 revealed that only 0.03% of the population had normal mucosa [3]. It was found that 1.7% of the population had superficial gastritis (SG) as its most severe lesions. 44.8% chronic atrophic gastritis (CAG), 33.0% intestinal metaplasia (IM), 20.1% dysplasia (DYS), and 0.4% GC. In an unpublished follow-up study, the relative risks of developing GC compared to the baseline rate from CAG were 16 for IM, 20 for mild DYS, and 52 for moderate or severe DYS. These data, along with the anatomic distribution of precursor lesions and incident gastric cancers, suggest a multistage process of carcinogenesis involving a progression of precursor lesions, as discussed by Correa [4]. The purpose of the present trial is to determine whether certain interventions can reduce the rates of progression among these precursor lesions or accelerate the rates of regression, and thus reduce the prevalence of DYS and/or GC.

The 13 rural villages in Linqu in which we are conducting the study range in size from 642 to 1845 inhabitants. Almost all adult villagers are farmers. Of those surveyed in 1989, 42% had no schooling [5], and the average annual income in 1993 was 434 yuan, which is very low by Chinese standards. This study population offers several advantages in addition to a high prevalence of precancerous gastric lesions. First, the population is stable, facilitating follow-up and administration of long-term interventions. Second, many in this population have participated in previous gastroscopic surveys and shown a willingness to undergo this procedure repeatedly; indeed, 85% of those who underwent endoscopy in 1989 as part of an initial survey agreed to be endoscoped again in 1994 in a follow-up survey. Third, observational studies conducted in these villages provide a rationale for the planned interventions. Finally, owing to long-standing relationships with researchers at BICR and NCI, the intervention trial enjoys the trust and support of the villagers and their village leaders, which promotes good compliance.

A leading hypothesis is that treatment of *Helicobacter pylori* (HP) infection can retard the progression of precancerous gastric lesions, but no data have been reported to indicate whether or not treatment for HP infection lowers the risk of GC or the prevalence of precancerous gastric lesions. Several lines of evidence support this hypothesis. GC rates correlate with the prevalence of HP infection internationally [6] as well as within regions of China [7]. A series of nested case-control studies [8–10] has shown relative risks for GC of nine

Table 1 Interventions

Factors	Dose	Duration	Source
Helicobacter pylori eradication[a]			
Amoxicillin	1 g bid	2 weeks	Astra
Omeprazole	20 mg bid	2 weeks	Astra
Vitamin/mineral supplement[b]			
Alpha-tocopherol	100 IU bid	39 months	Shanghai Squibb
Vitamin C	250 mg bid	39 months	Shanghai Squibb
Selenium[c]	37.5 μg bid	39 months	Shanghai Squibb
Garlic preparation			
Extract	400 mg bid	39 months	Wakunaga
Oil	2 mg bid	39 months	Wakunaga

[a] We gave this treatment only to subjects who had serologic evidence of HP infection at baseline. Three months after the initial 2-week course, we gave subjects a ^{13}C-urea breath test and, if evidence of HP infection persisted, we gave them a second 2-week course of treatment.

[b] We included beta-carotene (7.5 mg bid) in the vitamin and mineral supplement from December 1995 to May 1996, then discontinued it.

[c] We obtained the selenium from a yeast preparation.

or more among persons with antibodies to HP detected more than 15 years before the onset of GC [11]. In 1994, a Working Group of the International Agency for Cancer Research concluded that HP was a human carcinogen [12]. In Linqu, HP infection has been associated with the prevalence of precursor lesions, especially CAG [13].

The prevalence of HP increases with age in Linqu until about age 12. In our study population, the HP prevalence is 69% in women and 65% in men. Although HP infection aggregates in families in Linqu, we know little about its modes of transmission, and no data are available on the rate of reinfection following treatment for HP in Linqu.

We chose the combination of amoxicillin and omeprazole used in this study (Table 1) because an earlier pilot study of 110 subjects in Linqu showed this regimen to be well tolerated and effective. Nine of these subjects developed rashes, however, and several had mild gastrointestinal complaints. Based on a ^{13}C-urea breath test for urease activity in the pilot study, the HP eradication rate was 62% after 2 weeks of treatment (72% if we included only those who completed a full 14-day course). More effective regimens consisting of three or more agents have been proposed [14], but these combinations are usually given under close medical supervision to people with active ulcers, and they are associated with more side effects. We preferred to use a previously field-tested regimen that we could apply safely to the general population in the rural setting of Linqu and to re-treat those whose HP had not been eradicated initially.

Observational studies and intervention trials suggest that supplementation with vitamins E and C and selenium might retard the progression of precancerous gastric lesions. In a population at high risk for cancers of the esophagus and gastric cardia in Linxian County, Henan Province, a randomized intervention trial of nearly 30,000 subjects showed that participants receiving a mixture of beta-carotene, vitamin E, and selenium over 5 years experienced a reduction

of 13% in total cancer mortality and 21% in GC mortality [15]. Observational studies have consistently demonstrated lower levels of GC risk in persons with higher intakes of fresh fruits and vegetables [16]. A case-control study of GC in Linqu demonstrated lower risks among those with diets rich in carotene and vitamin C [17], and, in a survey of micronutrients in over 600 adults with known gastric histopathology in Linqu, levels of beta-carotene and vitamin C were lower in those with IM than in those with CAG [18].

Accordingly, the vitamin and mineral supplementation initially included beta-carotene in addition to vitamins C and E and selenium (Table 1). We included beta-carotene in the mixture in the first cycle of treatments that began in November 1995 but omitted it from all micronutrient preparations after May 1996. The Data and Safety Monitoring Committee (DSMC) decided to drop beta-carotene from the vitamin supplementation because of a public announcement in January 1996 that smokers in the Beta-Carotene and Retinol Efficacy Trial had a 28% increase in lung cancer risk [19]. This finding agreed with an 18% increase in lung cancer risk among smokers who received beta-carotene in the Alpha Tocopherol, Beta-Carotene Prevention Trial [20]. Although a third trial, the Physicians Health Study, revealed no effect of beta-carotene on the incidence of lung cancer [21], only 11% of participants in that study were current smokers. In view of the increasing prevalence of smoking in China and the lack of evidence from controlled trials that beta-carotene reduces cancer risks when used alone, the Committee decided to drop it from the supplement.

The rationale for including a garlic preparation consisting of Kyolic® aged garlic extract [22] and of steam-distilled garlic oil is based on epidemiologic studies in Linqu and elsewhere and on laboratory data. A case-control study of GC in Linqu indicated that persons in the highest quartile of intake of allium-containing vegetables (including garlic, garlic stalks, scallions, chives, and onions) had only 40% of the risk of those in the lowest quartile [23]. A case-control study in Italy found a similar decrease of GC risk in those with the highest garlic intake [24].

No controlled trials, however, have demonstrated the efficacy of garlic or its products in affecting carcinogenesis in humans. Because garlic and its various uses in cooking can produce a wide variety of potentially active substances, there is no consensus on the optimal type of garlic preparation. Diallylsulfide and diallyl polysulfides, which are present in steam-distilled garlic oil, have antibiotic activity that might suppress HP infection [25, Table 5.7]. Diallyl sulfide reduces the number of colorectal adenocarcinomas in mice exposed to dimethylhydrazine [26]. Researchers have reported other inhibitory effects of steam-distilled oils on carcinogen-induced tumorigenesis ([25], p. 180). Topical application of Kyolic aged garlic extract inhibits murine skin tumors induced by 7,12-dimethylbenzanthracene (DMBA) [27]. This extract will come into contact with gastric mucosa in the present study.

In this study, we employ a placebo-controlled, double-blind, randomized 2^3 factorial design to determine whether treatment with amoxicillin and omeprazole for HP, supplementation with vitamins C and E and selenium, or supplementation with a mixture of Kyolic® aged garlic extract and steam-distilled garlic oil reduces the prevalence of advanced precancerous gastric lesions.

METHODS

Organizational Aspects

The trial is a collaborative effort by investigators at NCI and BICR and is supported by consultants and a Data and Safety Monitoring Committee (see the Appendix). Westat, Inc. provides logistic and data management support.

Study Population

As part of our earlier epidemiologic study in 1989 [3], we transcribed all the names of residents aged 35–64 from village rosters in 14 villages selected at random from townships in Linqu. In the fall of 1994, we added to the roster the names of all subjects who had aged into the range of 35–39. We invited the 4326 rostered individuals to participate in a screening program with gastroscopy and gastric biopsy as part of the previous study. From this group, we excluded 210 subjects as too ill to participate because of bleeding disorders, cancers (except nonmelanoma skin cancer), heart failure, emphysema, renal or liver diseases, or other life-threatening illnesses. In addition, 226 subjects refused to participate, yielding a study population of 3890 potential subjects for the trial (Figure 1). Of these, 291 in one village participated in a pilot study in the spring of 1994 and were not eligible for randomization into the present study, leaving a potential study population of 3599 subjects who had undergone baseline gastroscopy with biopsies in 1994.

To qualify for participation in the current intervention trial, each subject had to sign an informed consent form in the summer of 1995, indicating willingness to participate in the 42-month study. In addition, we required baseline information on age, gender, and HP antibody status. A total of 39 subjects refused, and 56 were ineligible because they were no longer in the eligible age range of 35–69, no longer alive, or no longer accessible in their villages (Figure 1). In addition, we excluded 67 subjects for histories of allergy to penicillin or related antibiotics and another 26 because their baseline assay results for HP their antibody status were not available. A total of 3411 subjects remained for randomization (Figure 1).

Interventions

Treatment of HP Infection (Factor 1)

Subjects with serologic evidence of HP at baseline were eligible to receive amoxicillin (1 g twice a day) and omeprazole (20 mg twice a day) in three capsules (two 500 mg amoxicillin and one 20 mg omeprazole) to be taken twice daily (before breakfast and dinner) for 2 weeks (Table 1). Look-alike placebo capsules containing lactose and starch for amoxicillin and sucrose and starch for omeprazole were given to serologically positive controls and to all seronegative subjects. We gave all subjects a ^{13}C-urea breath test 3 months after treatment to determine whether HP had been eradicated. Subjects given amoxicillin and omeprazole initially who had continued evidence of infection after 3 months received a repeat course of treatment for 2 weeks unless they had previously developed rashes or other evidence of allergy to the initial treatment. To protect

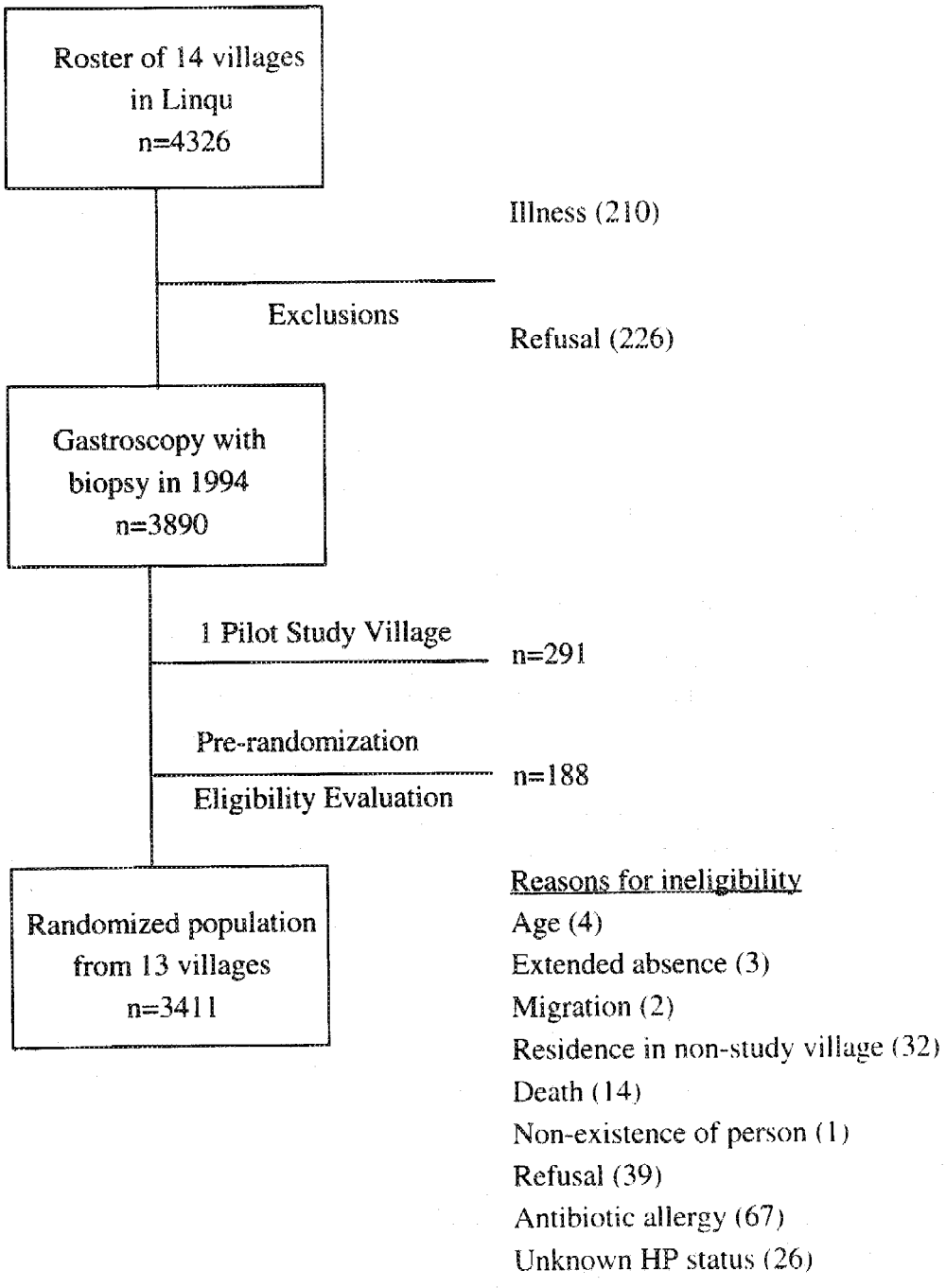

Figure 1 Identification of randomized participants.

the blinding, we selected randomly an equal number of subjects on the placebo arm from the same village and 10-year age range for re-treatment with placebo.

Vitamin and Mineral Supplementation (Factor 2)

Approximately 3 months after initial treatment for HP, supplementation with 100 IU alpha-tocopherol, 250 mg vitamin C, and 37.5 μg selenium twice a day (Table 1) began its 39-month course. Subjects receive this mixture in one capsule, to be taken twice daily before or after breakfast and dinner. From December 1995 to May 1996, this mixture also contained beta-carotene (7.5 mg twice a day). Look-alike placebo capsules contained cellulose, lactose, and magnesium stearate.

Garlic Preparation (Factor 3)

In this group, subjects take two capsules twice a day before or after breakfast and dinner. Each capsule contains 200 mg Kyolic aged garlic extract and 1 mg steam-distilled garlic oil. To prepare the extract, the manufacturer (Wakunaga of America, Co., Ltd, Mission Viejo, CA) slices garlic cloves and soaks them in aqueous ethanol (about 20%) for over 18 months at room temperature. The extract is then filtered, concentrated, and dried [22]. The extract is standardized to contain 1 mg S-allylcysteine but has other constituents also. To prepare the steam-distilled garlic oil, the manufacturer grinds garlic cloves in purified water and distills the mixture. The oil fraction is then microencapsulated by a spray-dry process that produces a powder of microencapsulated oil; 1 mg of garlic oil contains about 0.1 mg diallyl sulfide, 0.2 mg diallyl disulfide, 0.6 mg diallyl trisulfide, and 0.1 mg diallyl tetrasulfides and pentasulfides. The look-alike placebo capsules contain cellulose, granulated sugar, caramel, and magnesium stearate. Bottles holding placebo capsules contained minute quantities of garlic oil so they would smell like garlic.

Experiment Design

We divided the subjects into two main strata on the basis of whether they showed serologic evidence of HP infection at baseline (2285 subjects) or not (1126 subjects). We allocated treatments to seropositive subjects in accordance with a 2^3 factorial design to investigate the effects of the three factors. We allocated seronegative subjects in accordance with a 2^2 factorial design to study factors 2 and 3 and, to protect the blind, gave all seronegative subjects placebo capsules for amoxicillin and omeprazole.

We masked both the subjects and the researchers to treatment assignment. After confirming the eligibility of patients, we assigned treatments randomly at Westat, Inc. and used this assignment to distribute coded bottles of capsules from the pharmacy in the city of Weifang in Shandong Province.

To assure the balance of treatments by gender and age, we first classified eligible subjects into strata defined by HP antibody status and gender and then ranked them by age within these strata. We identified blocks of eight consecutive ages for seropositive subjects and four consecutive ages for seronegative

subjects and assigned the eight (or four) treatment combinations randomly in blocks.

Baseline Data

Between September and November 1994, we gave potential subjects physical exams, drew blood for HP serology, and interviewed each subject to determine his or her health, age, gender, socioeconomic status, diet, and willingness to undergo endoscopy with biopsies. We endoscoped willing and sufficiently healthy subjects and took gastric biopsies from seven standard sites.

Endpoint Assessment

We shall base the major endpoints on gastroscopy and gastric biopsies taken 42 months after randomization and after 39 months of dietary supplementation with factors 2 and 3. We shall take biopsies from seven standard sites: from midway between the cardia and angulus on the lesser curvature; from the middle of the greater curvature of the corpus; from the center of the angulus along the middle portion of the lesser curvature; from 1 cm from the pylorus along the lesser curvature; from the posterior wall of the antrum; from the anterior wall of the antrum; and from the greater curvature of the antrum. We shall assign each biopsy a histologic grade and severity score: 0 for normal, 1 for SG, 2 for mild or moderate CAG, 3 for severe CAG, 4 for superficial IM, 5 for deep IM, 6 for mild DYS, 7 for moderate DYS, 8 for severe DYS, and 9 for GC. We shall assign each subject a global histologic score and category equal to the largest score found on any of the seven biopsies. We shall assign any subject who develops symptoms and has a confirmed diagnosis of GC during the course of the study a global score of 9.

The major endpoints of the study will be (1) prevalence of DYS or GC (score ≥ 6); (2) prevalence of severe CAG, IM, DYS, or GC; and (3) average severity score.

Secondary endpoints include (1) rates of transition from baseline to final histopathologic states and the effects of treatments on these rates of transition; (2) evidence of the effectiveness of amoxicillin and omeprazole in eradicating HP, based on ^{13}C-urea breath tests 3 months following treatment, on annual serology, and on a final pathologic examination of biopsies to look for HP; and (3) blood pressure at the time of the final examination. In addition, we shall use pathologic data from ancillary biopsies used to assess rates of transition to GC. If regions outside the seven standard sites suggest GC on endoscopy, we shall take biopsies from those regions. We shall use the results from ancillary biopsies as well as the seven standard sites to diagnose GC as a secondary point.

Follow-up Procedures and Monitoring

To monitor toxicity from treatment with amoxicillin and omeprazole, we visited subjects on the active and placebo arms each day and asked them to report possible allergic or other reactions. We planned to discontinue treatment with amoxicillin and omeprazole if rashes or other allergic side effects or other serious toxicities occurred. During the subsequent 39-month phase of the trial

or supplementation with garlic and/or vitamins and selenium, we shall visit subjects every 2 weeks to detect toxicities. We immediately report any severe or life-threatening toxicity to the local health workers, the BICR, the NCI, and the DSMC. One member of the DSMC is located in Beijing and has the authority to break the treatment code for an individual subject if it becomes necessary.

To monitor treatment compliance, we visited subjects daily during the phase of treatment with amoxicillin and omeprazole, and we counted pills. We count pills monthly to monitor receipt of the garlic preparations and vitamin and mineral supplements. In addition, we obtain sera from 64 randomly selected subjects every 3 months, and we use assays for vitamins E and C to compare vitamin levels among subjects assigned to active supplementation with those among subjects not so assigned. Likewise, we conduct assays for S-allylcysteine to monitor receipt of garlic supplementation. In the first year, we collected these samples from four villages. Xin Zhuang, Guo Jia Zhuang, Hou Jia He, and Hou He Ye. Within each village and within each of the four treatment combinations based on factors 2 and 3, we collected sera from four randomly selected subjects. Sampling was performed with replacement every 3 months; thus, in principle, some subjects may be sampled more than once. In the second year, we shall sample four other villages and, in the third year, we shall sample the previous villages again. This pattern will allow an assessment of compliance in eight of the participating villages and will enable us to assess time trends in the first four villages studied.

Data Management and Quality Control

Detailed procedure manuals describe the tasks to be performed, ranging from the distribution of pills to gastroscopic and laboratory procedures. Investigators from BICR supervise field work in Linqu and at the Weifang Blood Center, where they maintain a medical data file on each study participant. Death certificates for deceased subjects and hospital records for incident gastric cancers are collected during the trial. Investigators at the Weifang Blood Center record compliance and toxicity data biweekly and use an automated study management system to monitor participation and completeness of data collection.

Data are entered twice into computer-readable media to detect errors. Automated field checks and logical crosschecks of data are performed by data managers at BICR and at the Weifang Blood Center, as well as by staff at Westat, Inc., who communicate with investigators at BICR and Weifang to resolve discrepancies.

Staff from NCI conduct annual visits to observe operations in the field and check quality-control procedures. We shall confirm all reports of intercurrent cancers and deaths by reviewing patient records.

Power and Other Statistical Considerations

Preliminary data are insufficient to help us estimate the likely magnitude of intervention effects. A cross-sectional survey in 1989, however, showed that the prevalence of severe CAG, IM, and DYS rose steadily with age, with increases per year of age of approximately 1.0% for severe CAG, 1.2% for IM, and 0.8% for DYS. Hence, over a 39-month period, the prevalence of DYS might increase by 2.6% in the absence of treatment. If intervention not only prevents

such progression but also induces a comparable amount of reversion from DYS to lesser grades, treatment might reduce the prevalence of DYS or GC by about 5%. If the prevalence of DYS in a control arm is 22% [3], a two-sided $\alpha = 0.05$ level test comparing two binomial proportions based on $3411/2 = 1705$ subjects in each arm would have power 0.96 to detect an absolute prevalence reduction of 5%. This calculation pertains to factors 2 and 3. Among the 2285 seropositive subjects receiving treatment for HP, the corresponding power is 0.88. If 20% of subjects do not receive endoscopic examinations in 1999, these two powers would decrease to .91 and .77, respectively. These calculations underestimate slightly the power of analyses based on logistic regression that also controls for age and baseline histopathology. Following Byar and Piantadosi [28], we do not adjust α levels to account for the fact that we test three main intervention effects, nor do we allow for the hypothetical reduction in power that would result if the interventions had negative interactions on the logistic scale [29].

For those who are seropositive for HP at baseline, the principal analysis will be a logistic regression of the prevalence of DYS or GC (severity score ≥ 6) on the main effects for the three interventions, on age, and on categories of baseline histopathology. For those who are seronegative at baseline, we shall perform a similar analysis but shall include only intervention factors 2 and 3. We shall obtain overall estimates of the effect of factors 2 and 3 by a weighted least-squares combination of estimates from the two serology strata. We shall conduct similar analyses using the prevalence of severity score ≥ 3 as an endpoint and shall use analogous procedures for multiple linear regression to analyze data on average severity scores. Based on a prevalence of 61% for a severity score of ≥ 3, the power to detect a 5% absolute decrease in prevalence is 0.84 for factors 2 and 3 and 0.68 for factor 1. We shall base secondary analyses of rates of transition on previously published methods [30].

It is possible that 39 months of supplemental vitamin and mineral or garlic will not be long enough to demonstrate the potential effectiveness of these interventions. For example, a recent study of the effects of supplementation with selenium revealed that treatment-associated decreases in mortality rates for several types of cancer are most pronounced after 10 years, although a tendency toward reduced risks appears within 3 years [31, Figure 3]. We shall evaluate the feasibility of extending follow-up and administration of the vitamin/mineral garlic interventions for 4 more years, until about 2003. Because pathologic assessment and analysis of the biopsies in 1999 will take more than a year, we shall need to decide whether to continue the supplements before we have endpoint data. If we obtain approvals and resources to extend the trial, we shall continue it for the additional 4 years unless the endpoint data indicate an adverse effect from vitamin and mineral or garlic interventions.

Laboratory Procedures

The final gastroscopic examinations will be conducted by experienced gastroenterologists and supervised by the Chief of the Department of Digestive Disease at BICR. A special training workshop will help standardize safety procedures, procedures to minimize the discomfort of patients, and methods of taking biopsies from standard sites. Subjects will receive a topical anesthetic but no sedation, and clinicians will examine and interview subjects to detect medical contraindications before proceeding with endoscopy. This team has performed 8000 consecutive endoscopies with biopsies of the seven standard sites without bleeding requiring transfusions or other life-threatening complications.

Pathologic diagnoses will be based on criteria proposed by the Chinese Association of Gastric Cancer [32]. Three senior pathologists at BICR will interpret each slide independently. Slides will be selected for blinded quality-control examinations by experts in gastric pathology in the United States and China.

For the enzyme-linked immunosorbent assay (ELISA), the HP antigen was prepared from two HP isolates from patients in Linqu [13]. The assay was positive if the ELISA optical density exceeded 1.0. Special studies with a similar preparation from five HP isolates from the United States [33, 34] and with a cut-point of 0.514 yielded an estimated specificity of 94.9% (37/39) based on 39 U.S. children who had no history of HP infection and who had had a negative direct urease assay, negative culture, and negative endoscopy and gastric biopsies [35]. The corresponding sensitivity for this assay in 132 Chinese subjects with biopsy-confirmed HP infection was 100% [36]. Because we used the cut-point of 1.0 in the present study, one might expect in this case a somewhat higher specificity and somewhat lower sensitivity.

To measure urease with the ^{13}C-urea breath test, we asked subjects to fast overnight, and then we collected baseline samples of exhaled CO_2. Each subject consumed 150 ml of a sweet starch paste to delay gastric emptying and then drank 10 mL of cold water containing 100 mg ^{13}C-urea (>99%) (Baylor Medical College, Houston, TX). We collected expired gas after 15, 25, and 50 minutes. We then treated approximately 20 ml of expired gas to extract CO_2 via a vacuum system for ^{13}C analysis by gas-isotope-ratio mass spectrometry (MAT 250, Germany) [37]. We computed the fractions of $^{13}CO_2$ compared to all CO_2 for the baseline and test samples and expressed them in parts per thousand. If the test sample fraction exceeded the baseline fraction by more than six parts per thousand, we regarded the test as positive.

We performed assays for vitamins C and E using previously published methods [38, 39]. The Wakunaga Company (Koda-cho, Takata-gun, Hiroshima Pref., Japan) analyzed levels of diallyl sulphide and diallyl polysulfides in the garlic pills using high-pressure liquid chromatography.

RESULTS

Study Population and Treatment Balance

The randomization achieved almost perfect treatment balance within strata defined by baseline HP serologic status and by gender (Table 2). Because of the mechanism of treatment allocation, the mean ages of active and placebo arms were very close within these strata (Table 2).

The study population consisted of 1753 (51.4%) men and 1654 (48.6%) women (Table 2). Among the 2285 HP antibody-positive subjects, 1143 (50.0%) were men. Among the 1126 HP antibody-negative subjects, 610 (54.2%) were men. The 13 contributing villages (and corresponding numbers of study subjects) were Hou Jia He (406), Suo Zhuang (398), Guo Jia Zhuang (361), Li Jia Gou (333), Xin Zhuang (284), Yang Jia He (267), Li Hu Zhuang (260), Xi Quad (247), Wang Jia Zhuang (242), Hou He Ye (182), Huang Ai Quad (168), Nan Yang He (151), and Xi Si Hou (112).

Table 2 Distribution of Treatments and Mean Age (SEM) in Strata Defined by Baseline HP Serologic Status and Gender

	HP Antibody-Positive				HP Antibody-Positive			
	Males		Females		Males		Females	
Treatment	Active	Placebo	Active	Placebo	Active	Placebo	Active	Placebo
Amoxicillin/ Omeprazole	571[a]	572	571	571	0	610	0	516
	47.08	47.15	46.80	46.78	—	48.25	—	47.25
	(.40)	(.40)	(.37)	(.37)	—	(.38)	—	(.41)
Viamins/Mineral	572	571	571	571	305	305	258	258
	47.14	47.09	46.79	46.79	48.25	48.24	47.24	47.26
	(.40)	(.40)	(.37)	(.37)	(.54)	(.54)	(.58)	(.57)
Garlic	571	572	571	571	305	305	258	258
	47.08	47.13	46.79	46.79	48.26	48.23	47.23	47.27
	(.40)	(.40)	(.37)	(.37)	(.54)	(.54)	(.57)	(.58)

[a] The top number is sample size, the middle number is mean age, and the bottom number is the standard error of the mean.

Table 3 Number of Persons with Adverse Reactions During Initial Treatment with Amoxicillin and Omeprazole or Placebo[a]

	HP Antibody-Positive		HP Antibody-Negative
	Amoxicillin/Omeprazole (n = 1142)	Placebo (n = 1143)	Placebo (n = 1126)
Rash	35 (3.1%)	1 (.1%)	3 (.3%)
Diarrhea	3 (.3%)	2 (.2%)	4 (.4%)
Abdominal pain	8 (.7%)	10 (.9%)	9 (.8%)
Abdominal bloating	3 (.3%)	4 (.3%)	1 (.1%)
Vomiting	5 (.4%)	0 (.0%)	0 (.0%)
Nausea	1 (.1%)	1 (.1%)	0 (.0%)
Heartburn	0 (.0%)	1 (.1%)	0 (.0%)
Headache	1 (.1%)	0 (.0%)	0 (.0%)
Other	3 (.3%)	1 (.1%)	1 (.1%)

[a] The total number of persons with adverse reactions was 85, whereas we indicate 97 adverse reactions in this table; 12 subjects had more than one adverse reaction.

Treatment with Amoxicillin/Omeprazole

The initial phase of HP treatment began on September 15, 1995, and ended on November 29, 1995, when the last village completed its 2 week course. Of 3411 randomized subjects, including 1126 seronegative subjects who received placebos, only 28 (0.8%) failed to take all their pills, as we determined by pill counts. Their reasons for not taking all pills were refusal (6), death (6), absence from town (6), rash (7), or other (3). Of the remaining 3383 subjects who took all their pills, 3050 (90.2%) took them on time in accordance with the protocol requiring twice-daily treatment. The remaining 333 (8.8%) took all their pills with some interruptions; of these, only 28 had delays of 1 day or more.

Subjects tolerated initial treatment with amoxicillin and omeprazole well (Table 3), and except for the occurrence of skin rashes, there was no statistically significant increase in the incidence of previously reported toxicities or side effects compared to subjects receiving placebo. We noted no life-threatening toxicities. Of 1142 subjects receiving active treatment, 35 developed rashes, compared to 1 of 1143 HP antibody-positive placebo controls ($p < 0.001$). Altogether, 39 subjects developed rashes during this phase of the trial. We discontinued treatment in 6 subjects. Although the protocol specified that subjects who developed rashes should stop taking amoxicillin and omeprazole, the remaining 33 continued to take their medicine or placebo without serious incident.

To determine the necessity of re-treatment, we administered ^{13}C-urea breath tests to all subjects. Of the group receiving active treatment, 36% had urease activity 3 months after treatment (Table 4). A much larger percentage of the placebo-treated seropositive controls had urease activity (87%), indicating that amoxicillin and omeprazole had a measurable impact on the prevalence of subjects with urease activity ($p < 0.001$). In addition, the prevalence of subjects with urease activity was 40% among those who were serologically negative for HP at baseline, which was indistinguishable from the prevalence among those receiving amoxicillin and omeprazole ($p = 0.078$). One interpretation of

Table 4 Results of ^{13}C-urea Breath Tests 3 Months after Initial Amoxicillin and Omeprazole or Placebo Treatment

	Seropositive for HP Initially		Seronegative for HP Initially
	Amoxicillin/ Omeprazole (n = 1,142)	Placebo (n = 1,143)	Placebo (n = 1,126)
Number (%) with positive ^{13}C-urea test	400 (36.1%)	974 (87.3%)	436 (39.7%)
Total number tested	1108	1116	1097

these data is that the urease test has low specificity and that amoxicillin and omeprazole have successfully reduced urease activity to the background levels found among uninfected subjects. To re-treat all subjects with possible residual infection, we used the original cut-off value (excess $^{13}CO_2$ fraction above 6 parts per 1000) for the urease test rather than increasing the cut-off value, which would have increased specificity but decreased sensitivity.

In May 1996, 386 subjects whom we had treated originally with amoxicillin and omeprazole, who remained urease-positive at 3 months, and who had not withdrawn from the trial or shown adverse reactions during the initial treatment with amoxicillin and omeprazole were re-treated for 2 weeks. We also re-treated 386 randomly selected placebo controls, matched on village, gender, and 10-year age group.

Among the 772 eligible patients in the second phase of treatment, 764 (99.0%) took all their pills. The reasons the remaining 8 did not take pills were refusal (2), absence from town (1), rashes (3), vomiting (1), and death (1). Of the 764 subjects who took all their pills, 676 (88.5%) took them on time in accordance with the protocol schedule. The remaining 88 (11.5%) patients took all pills with some interruptions, and of these, 19 had delays of 1 day or more.

No serious or life-threatening toxicities occurred during treatment, but one subject discontinued treatment because of vomiting and three others because of rashes.

Treatment with Vitamins/Mineral and Garlic

Dietary supplementation with vitamins and mineral (factor 2) and garlic preparation (factor 3) began on November 30, 1995. In December 1995, and in January, February, and March 1996, the proportions taking all required pills in each month were 94%, 86%, 90%, and 92%, respectively. From April 1996 through December 1996, the average monthly proportion of subjects taking all pills was 92.3%. Investigators have reported no toxicities in connection with these treatments.

Serum samples obtained from randomly selected subjects demonstrate higher levels of vitamins C and E in subjects assigned to factor 2 and higher levels of S-allylcysteine in those assigned to factor 3. During May 1996, the median concentrations of vitamin E were 1643 μg/dL and 825 μg/dL in the active and control groups, respectively ($p < 0.0001$ by the Wilcoxon test); the median vitamin C levels were 7.0 mg/L and 2.9 mg/L, respectively ($p < 0.0001$); and the median values of S-allylcysteine were 66 ng/mL and 41 ng/mL, respectively ($p = 0.013$).

Mortality

A total of 23 deaths occurred among cohort members through December 1996. Five occurred before treatments began (one from an accident, two from breast cancer, one from skin cancer, and one from stomach cancer). In principle, those who died of cancer would not have been eligible for randomization if their cancers had been diagnosed before randomization. Eighteen deaths occurred after treatments began; one person died as a result of drowning, two of emphysema, one of empyema, one of pneumonia, two of suicide, four of lung cancer, one of breast cancer, one of esophageal cancer, one of an accident, and four of cardiovascular disease.

DISCUSSION

The subjects in this study have participated enthusiastically and have complied excellently, as shown by pill counts and studies of serum levels of vitamins C and E and S-allylcysteine. We believe that most subjects will submit to endoscopic examinations in 1999, as they agreed to do when enlisting in the study. Many of them have undergone endoscopies in observational studies in 1989 and 1994, and they expect to benefit from endoscopic surveillance. Previous examinations led to the early detection of 27 gastric cancers. To encourage participation in the endoscopic examination in 1999, BICR will provide an expert medical team to offer advice and to help on a range of health-related issues at that time. BICR staff and village leaders will emphasize the importance of participation in the months preceding the examination.

Treatment with amoxicillin and omeprazole caused fewer gastrointestinal side effects than we had anticipated based on a pilot study and published data. Gastrointestinal complaints were no greater in the group taking amoxicillin and omeprazole than in the placebo controls. We noted rashes, however, in 3.1% of subjects receiving amoxicillin and omeprazole initially.

For the reasons described in the introduction to this article, the DSMC decided to eliminate beta-carotene in May 1996, 6 months after vitamin and mineral supplementation began. If the mixture of vitamins C and E and selenium retards the progression of precancerous gastric lesions in the present study, then beta-carotene may possibly not have been a necessary ingredient in the combination of beta-carotene, vitamin E, and selenium used in Linxian [15].

We faced difficult choices in selecting treatments suitable for a blinded, controlled trial in Linqu. For example, some workers advocate the use of allicin, which has strong antibiotic properties ([26], Table 5) and is produced when the enzyme allinase reacts with alliin, a component in raw garlic and garlic powder. It seemed impractical, however, to administer raw garlic in this trial, and we did not expect garlic powder capsules to yield significant quantities of allicin in the stomach, where acidic contents rapidly deactivate allinase [40]. Garlic powder may warrant study in its own right, because it contains many substances found in cooked garlic, which studies have associated with reduced GC risk [24]. Likewise, it would be worthwhile to study more intensive regimens to eradicate HP, including those with three or more agents and those involving repeated courses of treatment, but these regimens did not seem feasible or justifiable in the asymptomatic rural population of Linqu.

Future challenges include maintaining high compliance rates, obtaining complete and accurate data on final endoscopies and biopsies, and clarifying the

extent to which amoxicillin and omeprazole eradicate HP by analyzing serial data on antibody status and histopathologic evidence of HP infection in 1999.

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APPENDIX

Participating Institutions and Staff

Steering Committee

Joseph Fraumeni, Jr.	Co-Chair, Director, Division of Cancer Epidemiology and Genetics, National Cancer Institute, USA
Guang-wei Xu	Co-Chair, Director, Beijing Institute for Cancer Research, School of Oncology of Beijing

Naitonal Cancer Institute

Mitchell H. Gail	Principal Investigator and Co-Project Officer
Wei-cheng You	Principal Investigator and Project Manager
Linda M. Brown	Principal Investigator and Co-Project Officer
Steven D. Mark	Principal Investigator
Frank D. Groves	Pathologist
Charles S. Rabkin	Epidemiologist

Beijing Institute for Cancer Research and School of Oncology of Beijing Medical University

Guang-wei Xu	Principal Investigator
Yun-sheng Chang	Principal Investigator and Field Director
Lian Zhang	Chief Laboratory Scientist
Ji-you Li	Chief Pathologist
Mao-lin Jin	Chief Physician
Jun-ling Ma	Field Supervisor
Wei-dong Liu	Field Supervisor

Data and Safety Monitoring Committee

Abraham Nomura	Chairman
Mimi Yu	Epidemiologist
Jun-she Chen	Nutritionist
David Fleischer	Physician

Consultants

William J. Blot	Design
Martin Blaser	*H. pylori* testing
Pelayo Correa	Pathology
Pentti Sipponen	Pathology
Chung S. Yang	Nutrient assays

Supporting Institute

Linda Lannom	Westat, Inc.
John Heinrich	Westat, Inc.
Jason Hu	Westat, Inc.

Intervention Agents Preparation

Amoxicillin/ omeprazole	Ellick Wong and Anders Walan Astra (East Asia Region)
Garlic preparation	Takeshi Yamasaki and Harunobu Amagase Wakunaga of America Co., Ltd.
Vitamins/mineral	Michael Jahn, Weili Wu, and Joyce Chiu Sino-American Shanghai Squibb Pharmaceuticals, Ltd.